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AIM: The objective of this study was to determine the effect of dry herbal mixture and sunflower oil on fermentation end products, microbial population and concentration of fatty acid in rumen fluid using *in vitro* and *in vivo* methods.

INTRODUCTION: The high productivity rate in ruminant sector is associated with feeding of high-concentrate diets to ruminants. Feeding regimes with high content of concentrates can result in alteration of microbial fermentation processes in rumen. The beneficial effects of using medicinal plants as a part of the diet depends on the contained amount and sort of bioactive compounds (tannins, saponins, essential oils). The addition of medicinal plants together with dietary fats could affect the microbial population leading to an increase of polyunsaturated fatty acids in ruminant products and could also alleviate the negative effect of high-concentrate diets.

MATERIALS AND METHODS: Four rumen fistulated rams (Lacaune vs. Suffolk) were used in 4 × 4 Latin square design. The animals were fed a basal diet consisted of 720 g DM/day meadow hay and 540 g DM/day barley grain. Basal diet was enriched with no additive (control), a herbal mixture (HM, 10 % of meadow hay, 72 g DM/day), sunflower oil (SO, 44 g/day), and the combination of both (HMSO). Before the in vivo experiment, 24 h batch culture incubations were performed (control, meadow hay/barley grain; 400/600, w/w; HM, 10 % replacement of meadow hay; SO, 3.5 %DM; HMSO). The *in vitro* dry matter digestibility was determined from the difference in the substrate weight before and after incubation. The gas production was measured using pressure transducer technique. The concentration of methane, short-chain fatty acids and fatty acid methyl esters were determined by gas chromatograph (PerkinElmer Clarus 500, PerkinElmer, Inc., Shelton, CT, USA). Ciliate protozoa were counted microscopically. The values of pH were measured using pH meter (InoLab pH Level 1, Weilheim, Germany).

Results

Table 1 Effects of dietary treatments: control, herbal mixture (HM), sunflower oil (SO), and a combination of HM and SO (HMSO) on fermentation parameters and fatty acid concentration in rumen fluid *in vitro*

	Control	HM	SO	HMSO	SEM	<i>P</i> -value
Fermentation parameters						
IVDMD (g/kg DM)	579 ^b	655 ^c	465 ^a	584 ^b	5.4	0.001
Gas (ml/g DM)	135 ^{bc}	121 ^{ab}	149 ^c	114 ^a	4.8	0.001
Methane (ml/100 ml)	8.25 ^b	7.18ª	7.64 ^{ab}	7.19 ª	0.194	0.021
Short-chain FA (mmol/l)	48.7	36.2	44.3	42.2	2.36	0.149
Acetate (mol%)	67.1ª	68.3 ^{ac}	66.9 ^a	69.2 ^{bc}	0.45	0.015
Propionate (mol%)	16.6 ^c	15.6 ^b	16.6 ^c	14.2ª	0.14	0.001
n-Butyrate (mol%)	10.7 ^a	11.6 ^{ab}	11.1 ^{ab}	12.2 ^b	0.24	0.012
Total protozoa (x 10 ³)	199	162	190	187	14.2	0.618
Fatty acids (g/kg of FA)						
C _{16:0} palmitic	273 ^c	312 ^d	152 ^b	147ª	5.3	0.001
C _{18:0} stearic	450 ^c	431 ^c	372 ^b	325 ª	5.1	0.001
t11 C _{18:1} vaccenic	57.2 ^a	49.5 ^a	115 ^b	101 ^b	3.32	0.001
C _{18:2n6} linoleic	37.9 ^a	32.3ª	87.9 ^b	161 ^b	1.63	0.001
c9t11 C _{18:2} linoleic	2.20 ^b	1.35ª	4.51 ^d	3.62 ^c	0.115	0.001
C18:3n6 α-linolenic	18.8ª	14.6 ^a	24.6 ^b	29.7 ^c	1.03	0.001
Saturated FA	723 ^c	743 ^c	524 ^b	472 ^a	1.3	0.001
Monounsaturated FA	95 ^a	91 ^a	280 ^b	281 ^b	1.0	0.001

Herbal mixture



Acorus calamus L



Achillea millefolium L.



Urtica dioica L



Table 2 Effects of dietary treatments: control, herbal mixture (HM), sunflower oil (SO), combination of HM and SO (HMSO) on fermentation parameters and fatty acid concentration in rumen fluid of sheep

	Control	HM	SO	HMSO	SEM	P-value			
Fermentation parameters									
Short-chain FA (mmol/l)	60.2	51.2	58.1	51.0	3.82	0.499			
Acetate (mol%)	71.4	68.6	69.0	68.6	0.94	0.586			
Propionate (mol%)	14.6	16.2	15.8	16.4	0.64	0.542			
n-Butyrate (mol%)	10.9	11.2	11.6	11.0	0.62	0.980			
Total protozoa (x 10 ³)	248	217	188	160	42.1	0.619			
рН	6.53	7.05	6.81	6.70	0.157	0.506			
Fatty acids (g/kg of FA)									
C _{16:0} palmitic	314 ^b	290 ^{ab}	239 ^a	228 ^a	13.7	0.021			
C _{18:0} stearic	415 ^a	436 ^a	534 ^b	570 ^b	19.4	0.009			
t11 C _{18:1} vaccenic	32.6	32.7	40.8	36.5	4.05	0.747			
C _{18:2n6} linoleic	66.4 ^b	65.6 ^b	39.7 ^a	38.1ª	3.06	0.001			
c9t11 C _{18:2} linoleic	0.41	0.77	0.52	0.69	0.128	0.069			
C _{18:3n6} α-linolenic	26.2 ^b	29.5 ^b	19.5 ^a	13.8ª	2.22	0.001			
C _{18:3n3} γ-linolenic	2.06 ^b	2.02 ^b	1.41 ^a	1.32ª	0.124	0.005			
Saturated FA	810 ^a	808 ^a	848 ^b	863 ^b	9.1	0.012			
Monounsaturated FA	93	92	88	81	8.3	0.679			
FA, fatty acids. Within a row , means without a common letter (a, b, c) differ at <i>P</i> <0.05.									

DM, Dry matter; IVDMD, *in vitro* dry matter digestibility; FA, fatty acid; Within a row,, means without a common letter (a, b, c) differ at *P*<0.05.





PUFA, polyunsaturated fatty acids. Means without a common letter (a, b, c, d) differ at *P*<0.05.





Calendula officinalis L.



Cichorium intybus L.



Fig 2 Effect of dietary treatments: control, herbal mixture (HM), sunflower oil (SO), combination of HM and SO (HMSO) on the concentration of PUFA in rumen fluid of sheep



PUFA, polyunsaturated fatty acids. Means without a common letter (a, b) differ at *P*<0.05. **CONCLUSIONS:** Addition of HM and HMSO to the diet positively affected the fermentation and fatty acid composition of rumen fluid after 24 h *in vitro* incubations, however these results were just partly confirmed under *in vivo* condition. Result from these experiments can point to the promising beneficial effects of using a herbal mixture in the high-concentrate diet of sheep.

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